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THESIS

FORMALDEHYDE
 AS A STIMULANT
 TO PLANT GROWTH

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FORMALDEHYDE AS A STIMULANT TO PLANT GROWTH

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1. Introduction.

The action of formaldehyde as a stimulant to plant growth revives the so-called law or hypothesis of Arndt (1)** that substances toxic to the living plant may, in very dilute concentrations, act as a stimulant. In general, between the toxic and stimulative concentrations there is the gradation of the former into the latter. This gradation may properly be called "toleration". (See plate 1.)

That a very small quantity of formaldehyde (1 to 20,000) is intensely poisonous to plants is set forth by Bokorny (2). This poisonous factor is well known and practical use is made of it in processes of disinfection and preservation. That it can act directly as a stimulant is the object of the experimental work of this paper. Indirect stimulation is well known, but little or no attention has been paid to the direct influence of this chemical on the growth of the plant.

There has been done considerable experimental work with formaldehyde in its relation to the plant, but the greater num-

*This study was suggested to the writer by Professor A. Vincent Osmun, Head of the Department of Botany of the Massachusetts Agricultural College, Amherst, Massachusetts. Professor Orton L. Clark, in charge of the Plant Physiology courses, made many valuable suggestions. To both of these teachers the writer wishes to express his sincere appreciation.

**Consult Bibliography.

ber of the studies has been made with the green parts of plants. These attempts have been made in the direction of establishing the intermediate synthetic compounds between carbon dioxide and the starches through photosynthesis, the formaldehyde being present in the atmosphere in connection with sunlight.

The object of the following experiments was to ascertain if formaldehyde, in weak solutions, would in any way stimulate root growth. If such a stimulation could be determined it is only natural to suppose that, because of the increased feeding area thus obtained, the green parts above would be proportionally greater than untreated plants.

II. Review of the Literature.

Many have noted that where soil is treated with formaldehyde as a sterilizing agent, the subsequent growth exhibits a seeming stimulation as compared with the untreated soil. Apparent stimulation has been obtained with lettuce from soil treatment with formaldehyde at the Massachusetts Agricultural Experiment Station (3). Formaldehyde has been advantageously used for the partial sterilization of soils for experimental purposes as well as for commercial gardening. Buddin (4) has found that "Consistently good results have been obtained in pot experiments from the treatment of soils with formaldehyde for the growth of tomatoes, the crop obtained being both earlier and greater in bulk." Buddin, in attempting to explain his positive results, has induced his readers to believe that the increases are due to the relation of the chemical to the flora of

the soil. His conclusions are derived from the numbers of bacteria, protozoa, and the amount of ammonia and nitrate present in treated and untreated soils. Thus, he has obtained an indirect benefit, for the flora of the soil influenced the amount of the available nutritive substances in the treated soil to such an extent that a marked increase was obtained over the untreated soil.

Grafe and Portheim (5) made studies with and without formaldehyde gas under bell jars on various seedlings and twigs, the experiments running from 7 to 16 days. A stimulating effect was obtained as far as the epicotyl and primordial leaves were concerned with the gas; but the hypocotyl and internodes were better without the gas. With the gas the roots were decidedly shorter and less developed than when grown under normal conditions. These investigators conclude that formaldehyde acts either as a stimulant or food to organisms bearing chlorophyll. Here, for the first time, we find an allusion to the effect of formaldehyde on roots; but the formaldehyde was used in the atmosphere and not in solution.

Miss Baker (6) found that formaldehyde seemed to stimulate respiration in the dark. In the light she found evidence that it was assimilated.

Benedicenti and de Toni (7) have made extensive researches and contend that formaldehyde is highly injurious to plant life. It is true that they obtained a stimulus which was measured by increased respiratory activity but later this fell greatly below the normal and the detrimental effect on the plant could almost

be measured directly from the amount of formaldehyde used and the duration of the treatment. As in the work of other investigators these men introduced the formaldehyde into the atmosphere.

That formaldehyde is highly toxic in comparatively small concentrations is well known. Hence, it has widespread use for disinfection purposes. This highly toxic property lends its use to the partial sterilization of soils. Used at the rate of 1 to 100, a gallon per square foot, it is so toxic to plant growth that 4 to 7 days must elapse before seeds are planted or a crop set. That formaldehyde will be retained by the soil for a fairly long time is proved by some of Buddin's (4) experiments. According to his results "Schiff's reagent showed that with the higher doses some remained in the soil throughout the experiment and kept the soil in a practically sterile condition." These higher doses mentioned refer to M/10, M/50, and M/100 concentrations of formaldehyde. The length of one experiment was 90 days, and the duration of the second experiment was 170 days. The solution 1 to 100 corresponds to an M/7.5 concentration. Because the soil retains formaldehyde for such a long time it appears that there is some connection between this factor and the increased vigor of the plant.

As yet no one has definitely concluded whether it is a stimulative or a nutritive effect caused by the formaldehyde. It can possibly be both: the former through the effect on enzymes, and the latter through the aid of radiant energy.

Seligman (8) has learned that formaldehyde increases the activity of three oxidizing enzymes in milk. Bokorny (9) made some studies with the effect of formaldehyde on protoplasm and enzymes, protoplasm being readily killed with a 0.01% solution, but it took a 0.5% solution to destroy the efficiency of malt diastase. This author claims that, in general, formaldehyde is a poison for enzymes as well as for the organism, but did not take into consideration the purity of the preparation or protective substances.

The writer has not as yet carried out any experiments with the weak concentrations acting on various enzymes.

There are many different figures in regard to the toxicity of formaldehyde to the living plant, and the following experimental work rather sets forth the idea that it is the nature of the plant under treatment and its condition that are the vital factors. Thus, Bokorny (2) states that formaldehyde 1 to 20,000 is very toxic, while Treboux (10) claims that Elodea can live perfectly well in a 0.0005% solution which is ten times stronger than the 1 to 20,000 concentration.

This review of the literature shows that mainly speculative work has been done with formaldehyde in regard to direct stimulation. The above-named investigators have dealt more with formaldehyde and photosynthesis, and when an author has noted a stimulus no direct expression as to its cause has been set forth. With the ever increasing use of this chemical in agricultural practice it is very desirable that its effect on the plant be more thoroughly studied.

III. Experimental

The formaldehyde used in the following experiments is the ordinary commercial product which carried approximately 37% formaldehyde. As the writer was not after strictly quantitative results the formaldehyde was figured as 40% in order to facilitate the preparation of the dilutions used. Hence all of the solutions are approximate, yet the dilutions are so great that fairly definite conclusions may be made.

The various concentrations of formaldehyde used in these experiments were most conveniently prepared according to the following plan. Figuring the formaldehyde (HCHO) as 40%, one cubic centimeter of such a solution would contain 0.4 grams HCHO . Fractional molar solutions were used. A molar solution of HCHO contains 30 grams in 1,000 cubic centimeters of water.

The solutions used are the outcome of experiments to determine the degree of tolerance and the extent of the stimulus. They represent fourteen solutions in all, running from $M/10$ to $M/200,000$. In the following table the weight of formaldehyde per liter is recorded for the benefit of those readers who might be interested in the approximate amounts of HCHO present.

Method for preparing the solutions.

Stock Solution A = 1 cc. HCHO and 9 cc. H₂O (1 cc. = 0.04 gms. HCHO)
 " " B = 1 cc. HCHO and 19 cc. H₂O (1 cc. = 0.02 " ")
 " " C = 3 cc. Stock B and 17 cc. H₂O (1 cc. = 0.003 gms. HCHO)
 " " D = $\frac{1}{2}$ cc. HCHO in 2,000 cc. H₂O (1 cc. = 0.0001 gms. HCHO)

Solution No.	Molar Solution	Grams HCHO per liter	
1	M/10	3.00	992.5 cc. H ₂ O and 7.5 cc. HCHO
2	M/50	0.60	998.5 " " " 1.5 " "
3	M/100	0.30	992.5 " " " 7.5 " Stock sol. <u>A</u>
4	M/200	0.15	996.25 " " " 3.75 " " " "
5	M/400	0.075	996.25 " " " 3.75 " " " <u>B</u>
6	M/600	0.05	997.5 " " " 2.5 " " " "
7	M/800	0.0375	987.5 " " " 12.5 " " " <u>C</u>
8	M/1000	0.03	990.0 " " " 10.0 " " " "
9	M/5000	0.006	940.0 " " " 60.0 " " " <u>D</u>
10	M/8000	0.00375	962.5 " " " 37.5 " " " "
11	M/10,000	0.003	970.0 " " " 30.0 " " " "
12	M/50,000	0.0006	994.00 " " " 6.0 " " " "
13	M/100,000	0.0003	997.0 " " " 3.0 " " " "
14	M/200,000	0.00015	998.5 " " " 1.5 " " " "
15	Check	None	1000.0 " "

Study with two-day old radicles of peas (*Pisum sativum*)

This constituted the major portion of the work, but the results are not as striking as in following experiments. This study was undertaken with the object to determine if there was a stimulation by formaldehyde on the non-green parts of plants, all factors of a photosynthetic nature being obviated.

That formaldehyde can act as a food was found to be true by Bouilhac (11), who found that the algae *Nostoc* and *Anabena* were able to polymerize formaldehyde, but in order to do so sufficient sunlight must be supplied, and this must equal that under which these algae naturally grow. Bokorny's (12) experiments show that in sunlight *Spirogyra* with a 0.001% solution of formaldehyde gave a strong positive result, proved by a very rich starch formation.

The work of these scientific investigators has resulted in establishing proof that the green parts of living plants are able to polymerize formaldehyde in the presence of sunlight.

It was, therefore, decided to work in a preliminary manner with the non-green parts of plants, before chlorophyll had an opportunity to form. This necessitated the use of a dark room with the exclusion of all light. Weak solutions of formaldehyde must be used, for it was found out that the solutions had a deleterious action in many instances. The M/1000 is probably the last solution in the series to show a distinct toxic action. This solution contains 0.03 grams of formaldehyde per liter and represents a 0.003% solution.

The experiments on the radicles were carried out in a series of battery jars, each jar having nearly a four liter capacity. The solutions used were prepared according to the previously mentioned dilution method. Two liters of a solution were used in each jar. Twenty-five seeds were supported on floats of paraffin, 15 centimeters in diameter, $\frac{1}{2}$ centimeter thick, with twenty-five perforations, $\frac{1}{2}$ centimeter in diameter. The average length of time for each experiment was 15 hours, in a dark room, with a temperature that did not vary more than one degree. It might be well to mention here that the temperature varied for the series of experiments but not more than a degree for any one. Perhaps this had something to do with the variation in the results, but the writer feels that the variations were caused by the condition of the seeds. The radicle before going into the solution should be marked at a suitable distance back of the growing point.

Much trouble was encountered in obtaining a good type of root. A straight radicle is necessary in order to make accurate measurements. Root-hairs must not be in evidence, for these interfere in marking off a definite length. Seeds of Pisum sativum were selected to work with, because the root-hairs appear on this seedling much later than on the others tried.

Starting these seedlings upon moist filter paper was a failure, since the resulting radicles were curved due to tropic responses. Growing them in moist sand and sawdust with the radicle pointed down, in order to obtain the best advantage of

geotropism, was not a success, because a contact stimulus occurred that caused swellings and the production of root-hairs. The most successful method tried is to sprout the seeds on galvanized wire of $\frac{1}{4}$ inch mesh over a large culture dish. The procedure in detail is as follows:- The seeds of Pisum sativum are immersed for 10 to 15 minutes in a solution of mercury bichloride 1 to 1000, washed in distilled water, and soaked for 10 to 12 hours in distilled water. This disinfection process is necessary to destroy bacteria and fungi, especially Rhizopus nigricans, which makes a rapid growth if the treatment is not applied. All the working apparatus used in connection with this method must be as nearly sterile as possible. Galvanized wire of $\frac{1}{4}$ inch mesh is placed over a culture dish half filled with distilled water. The seeds, after soaking, are carefully placed in such a position that the radicle shall pass through the center of the mesh, care being taken not to crowd the seeds. They are covered with moist cotton and the whole covered with a bell-jar which is protected from the light by means of a black cloth covering. These directions must be closely adhered to, for if the radicle touches the wire a contact stimulus overcomes geotropic influences to such an extent that the radicle will even make a complete turn or circle. Should the seeds be placed in a wrong position gravity will so twist the radicle that the seeds will be useless for the experiment. Phototropic influences vary the normal growth, and even hydrotropic curvatures must be considered. If there is an insufficiency of water in the culture dish the radicle will penetrate only

as far as the moist vapor hangs from the cotton. Too much water causes a thin radicle with aerotropic curvatures. The seeds are ready to use in 60 to 70 hours from their first treatment at which time they should have a straight radicle $1\frac{1}{2}$ to 2 centimeters in length.

The results of these experiments on the effect of formaldehyde on the radicles of peas were not as satisfactory as expected, except that a stimulation was shown. In several cases this stimulation was over 50% and even went as high as 100%. These percentages were obtained by comparing the increased growth with that of the check. Nearly 50 trials were made with the hope that a mathematical curve could be plotted from the results, beginning with the solution with the least toxic effect (M/5000) and rising to the greatest stimulating solution (M/50,000) and dropping with the succeeding weaker concentrations.

The seeds, after 15 hours, were taken from the paraffin floats and the radicles accurately measured from the India ink mark, which was well behind the growing point. The difference between the original and final distances represents the increase in length, the average of which was taken for the final results.

Considering the check equal to 100 the comparative relation of the effect of the different concentrations of formaldehyde with the check is as follows:

Check	100
M/800	72
M/1000	85
M/5000	102
M/8000	102
M/10,000	93
M/50,000	128
M/100000	103
M/200,000	107

As yet there has been no reason to explain why there appeared a decrease in the M/10,000 series. Such a decrease appears in later experiments dealing with the effect of formaldehyde on cuttings, though not necessarily occurring in the M/10,000 concentration. Discounting this variation and considering that a stimulation has been shown within the 15 hours we can readily comprehend what the effect will be on the weight of a plant if this stimulation continues. As yet no experiments have been undertaken to try out stimulation studies on the non-green parts for more than 15 hours. It is expected that later work will prove that the stimulation is continuous.

Herbaceous cuttings treated for 24 hours.

On exposing herbaceous cuttings of Tradescantia zebrina to the various concentrations for 24 hours and then transferring them to tap water for a week the effect of formaldehyde as a stimulant under these conditions was studied. Ten cuttings each having three nodes exposed were used in each concentration. Care was taken that each cutting was washed before being put into the tap water. A slight stimulation was noted after M/1,000. The results are in the following table.

No.	Concentration	Gms. HCHO per liter.	1	2	3	Days 4	5	6	7
1	M/10	3.00	Wilted	-	Dead	-	-	-	-
2	M/50	0.60	Wilted	-	-	-	-	-	Dead
3	M/100	0.30	Roots did not form at exposed nodes, but at the top on 4th day, so no wilt.						
4	M/200	0.15	-	Roots ^D * 1 mm. (did not lengthen.)					
5	M/400	0.075	-	Roots ^C 1½ cm.					
6	M/600	0.05	-	Roots ^C 1½ cm.					
7	M/800	0.0375	Roots ^C	-	3 cm.				
8	M/1,000	0.03	Roots ^C	-	3 cm.				
9	M/5,000	0.006	Roots ^A	-	4 cm.				
10.	M/8,000	0.00375	Roots ^A	-	4 cm.				
11	M/10,000	0.003	Roots ^A	-	4 cm.				
12	M/50,000	0.0006	Roots ^A	-	4 cm.				
13	M/100,000	0.0003	Roots ^B	-	½ cm.				
14	M/200,000	0.00015	Roots ^B	-	½ cm.				
15	Check	-	Roots ^B	-	½ cm.				

* Refers to length of root: A = —, B = —, C = —, D = .

The number of roots per concentration also signifies a stimulation.

No. of concentration	No. of roots	No. of concentration	No. of roots
1	0	9	86
2	0	10	88
3	0	11	93
4	0	12	77
5	70	13	87
6	77	14	88
7	70	15	72
8	81		

Dormant cuttings treated for 24 hours.

Dormant wood cuttings immersed for 24 hours in the various concentrations of formaldehyde (M/10 to M/200,000) did not show any appreciable effect of the treatment. The cuttings were Salix fragilis which roots readily, roots appearing on all series in 10 days. None of the series showed any marked difference, all producing roots, and none showing that the stronger concentrations had any deleterious effect.

Herbaceous cuttings in weak concentrations of formaldehyde.

When formaldehyde in weak concentrations is used continuously with Tradescantia some very striking results are obtained. In this and other experiments where the green parts are allowed to remain we must consider the possibility of the polymerization of the formaldehyde which would make this chemical available as a source of energy.

The Tradescantia cuttings used were trimmed so that all had practically the same leaf area. Two cuttings were used to each solution in large test tubes. Two series were used: (a) unrooted cuttings, (b) rooted cuttings, 11 days old. The duration of the experiment extended over a period of 7 weeks during which time the solutions were daily turned out and renewed. The same concentrations were used as in the previous experiments. Excellent evidences of toleration were obtained as shown by root development. A stimulation effect also appeared and was evidenced by more than one character. In this experiment it is deemed worthy of note to mention the manner in which the leaves died in the more concentrated solutions. After 24 hours the leaves on the cuttings in the M/10 solution showed a wilting, water-soaked appearance, with a blackening on the veins and branches of veins. These symptoms also appeared the second day on those in the M/100 solution, and on the tenth day in the M/200 solution. These latter two showed practically no blackening. To determine the selective power of the plant, or the probability that the formaldehyde solution can be carried to all parts of the plant by the vascular system, the following experiment was made:- A cutting of Tradescantia which had been

in the dark 24 hours was placed in an M/10 solution and kept in a dark room for another 24 hours, after which time the leaves appeared wilted and blackened, water-soaked areas having appeared along the main vascular system, beginning at the stem end and working toward the tip and sides. That formaldehyde has been conducted to the leaf may be proved by using the following qualitative test for HCHO recommended by Schryver (13). The leaf is thoroughly crushed in water and made up to 10 cc. Add 2 cc. of a 1% solution of phenylhydrazine hydrochloride freshly made up and filtered; add 1 cc. of a 5% solution of potassium ferricyanide freshly made up and then 5 cc. of hydrochloric acid. A magenta color is the test, which was very pronounced in this case. The cutting is exposed to the HCHO in the dark because various authors, as reviewed by Polacci (14), have found HCHO present in the leaves. It was found that 24 hours in the dark would remove any traces of this.

The roots of the cuttings in the M/400 and M/600 solutions were very short with no root hairs. No roots appeared on the cuttings in solutions stronger than M/400. The roots on the M/800 and M/1000 were slightly longer with very short root hairs. The roots on the M/5000 cuttings did not appear stunted, yet the root hairs were short.

The secondary roots appeared in 18 days on the M/5000 and weaker solutions. None appeared on stronger concentrations. These secondary roots showed a variation fairly consistent in both sets.

A represents best development of secondary roots.

D represents poorest development of secondary roots.

	Unrooted cuttings	Rooted cuttings
M/800	-	D
M/1000	-	C
M/5000	D	D
M/8000	C	C
M/10,000	C	C
M/50,000	B	B
M/100,000	C	C
M/200,000	A	A
Check	C	B

The rooted cuttings followed nearly the same development as the unrooted cuttings. After they had been subjected to the formaldehyde solutions for 3 days the roots and root hairs turned brown in the concentrations down to M/5000, the last concentration to evidence any toxic effect. In the final examination it was found that the cuttings exposed to the M/5000 dilution and weaker had a very marked increase in root development as compared with the check. (See plate 2)

There was not much difference in the new leaf area formed except in one place. The check averaged 5 new leaves and the M/10,000 set averaged 6 leaves. The other sets produced 4 leaves. The cuttings in the M/10,000 concentration in both the rooted and unrooted sets were by far the best in the experiment. Not only did they have a greater area of root development but the green parts were longer and larger. (See plate 3)

Though stimulating results are very apparent, the writer wishes to be taken in to consideration the possibility of an increased food supply due to the presence of formaldehyde, aided by sunlight.

Dormant cuttings in weak concentrations of formaldehyde.

To try the effect of these weak concentrations of formaldehyde on dormant cuttings, the following experiment was undertaken:- Cuttings of willow (Salix fragilis) were chosen because they readily send out roots when placed in water. These cuttings were taken February 20, 1919. Three cuttings were used in each solution which was daily changed. All the series produced leaves, but the cuttings in the M/10 and M/50 concentrations failed to produce roots. However, the green leaves of these two series did not last more than three weeks, the typical formaldehyde blackening appearing which was later followed by the dropping off of this foliage. The stems of these two series also blackened under the surface of the solution. The injury first began around the lenticels and then gradually spread.

The roots which formed in the M/400 concentration were quite short, but very thick and dark. (See plate 4)

Two days after the roots appeared a record was made showing the comparative lengths. A week later a similar record was made. (See following table) After seven weeks the roots were removed, air-dried, and weighed. (See table)

Table showing record of willow cuttings in weak concentrations of formaldehyde for seven weeks. A = greatest length of root; F = the least.

Molar Concentration	Roots two days old	Roots nine days old	Dry weight of roots in gms.	Comparative Check = 100
M/10	-	-	-	-
M/50	-	-	-	-
M/100	F	E	0.005	6
M/200	F	E	0.009	10
M/400	E	F	0.017	19
M/600	D	D	0.058	66
M/800	DD	C	0.105	119
M/1000	C	B	0.085	97
M/5000	B	B	0.093	106
M/8000	A	A	0.081	92
M/10,000	B	B	0.082	93
M/50,000	B	B	0.068	77
M/100,000	B	B	0.083	94
M/200,000	B	B	0.095	108
Check	C	D	0.088	100

The above table shows some signs of a stimulating effect by the formaldehyde, but here again it is impossible to plot a mathematical curve. Furthermore, there is no direct relation between the dry weights and the length of the roots after a four-week interval.

The toleration as compared with the tolerance of the herbaceous cuttings and pea radicles is much different with these dormant cuttings. Toleration here ceases with the M/600 solution. With the pea radicles and herbaceous cuttings there was a toxic effect in the M/1000 solution.

Another significant fact is brought out in a second series of willow cuttings taken on March 20, 1919, or one month later than the first set. The cuttings taken in February produced roots in a concentration equal to M/100. Those cuttings taken in March did not produce roots until the concentration had been reduced to M/400. These cuttings were taken from the same tree. It is thus shown that as there exists a difference in the toxic effect of formaldehyde on different families, there also exists a difference in toxicity in regard to the cuttings of one species taken from the same plant at different times of the year.

Pea seedlings in formaldehyde and nutrient solution.

An experiment covering six weeks was made with pea seedlings in the various concentrations of formaldehyde. At the

end of two weeks Crone's (15) nutrient solution was added with the formaldehyde to supply the essential elements. The seedlings were started as previously described for the preparation of radicles. At the end of a week they were placed in pint jars supported by a perforated tile cover. These seedlings gave a very fine expression of toleration. (See plates 1 and 5) There was very little difference in the time of blossoming. M/200,000 bloomed two days in advance of M/5000 and M/100,000, and four days in advance of the remaining concentrations above M/1000. The tops and roots were cut, air-dried, and weighed. (See the following table)

Table showing the weight in grams of the air-dried tops, roots, and total of an average plant in formaldehyde plus nutrient solutions.

M/concentration	Tops	Roots	Total
M/10	-	-	-
M/50	-	-	-
M/100	-	-	-
M/200	.095	.038	.133
M/400	.116	.39	.155
M/600	.222	.051	.273
M/800	.182	.053	.235
M/1000	.170	.048	.218
M/5000	.541	.166	.707
M/8000	.386	.181	.567
M/10,000	.382	.178	.460
M/50,000	.418	.216	.634
M/100,000	.423	.200	.623
M/200,000	.569	.240	.809
Check	.603	.280	.883

The check produced the greatest weight for both top and root. From this experiment it appears that formaldehyde is toxic even in as weak a concentration as M/200,000. The figures with the M/5000 concentration are rather striking, for they show a great increase over the preceding and succeeding solution. Another noticeable feature connected with the solutions above the M/1000 was the fact that the roots were longer but were not as thick as those of the check. From M/600 to M/1000 the roots were very short but they were very numerous.

It was hoped that this experiment might prove that the action of the formaldehyde stimulus was continuous, but no such results were obtained in this case. However, we must bear in mind that the plants were in a solution which contained the essential elements in great abundance. Had they been grown in the soil it is possible that the stimulus obtained in previous experiments might have shown itself in a different manner. It has been noted elsewhere in this paper that formaldehyde produces more roots, longer root hairs, and longer secondary roots than untreated plants. This being the case, it is natural to suppose that a plant could establish itself more quickly in the soil.

As Benedicenti and de Toni (7) have noted, formaldehyde in the atmosphere may exert a stimulus, but a reaction follows which causes the plant to fall behind the untreated check. In the soil the results may well be different, for even if there is a reaction the plant through its greater root system has a better opportunity for taking up nutrients than the untreated

plant. Further experimental work is expected to give data showing a positive direct benefit.

IV. Conclusions

1. These experiments show that a positive stimulus may be obtained by using formaldehyde in very weak concentrations.

2. Stimulation may be obtained in the dark as is shown by the experiments with the two-day-old radicles of peas. This is contrary to what Grafe (16) found. His results show that formaldehyde in the air could be tolerated in the light up to a large percentage, but became toxic in darkness or on the colorless parts of plants. The stimulus obtained would naturally suggest that the formaldehyde had increased the activity of the enzymes to make the reserve food of the cotyledons more quickly available. Seligman (8) has noted that formaldehyde does increase the activity of certain enzymes. Grafe (17) claims that formaldehyde inhibits enzyme action, especially zymases and amylases.

3. Formaldehyde increases the root growth as proved by the experiments with the herbaceous cuttings of Tradescantia and the dormant wood cuttings of Salix. This is a direct stimulus and analogous to the indirect stimulus obtained by Buddin (4) through his improved condition of the soil flora.

4. These experiments show that in the stronger concentrations the roots were very short and numerous, while in the weaker concentrations the roots were much longer than the check, but their dry weight was less than that of the check. This statement does not coincide with the results from the experiment on dormant cuttings, for here in some instances the dry

weight of the treated cuttings was greater than that of the check.

5. The formaldehyde has a different effect on dormant cuttings taken in different months of the year. This difference is noted in tolerance rather than stimulation.

6. Toxicity, toleration, and stimulation are all abnormal conditions. A study on stimulation is only successful when the results are taken before a reaction sets in. There is no ideal way to measure stimulation, for a specific stimulus of one character is usually accompanied or followed by a specific negative stimulus of another character. Increased vigor of a plant grown in soil treated with formaldehyde may, possibly, be the visible result of the greater advantages derived through increased feeding area, due primarily to stimulated root development.

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Pea seedlings subjected to the various concentrations of formaldehyde, showing toxicity, toleration, and stimulation of roots. M/10 - top left. Check - lower right.

Plate 2.



Cuttings of Tradescantia after 7 weeks in the various concentrations of formaldehyde. M/200 at the extreme left, decreasing down to M/200,000 with the check at the extreme right. Set marked 2 = M/5,000 and following sets show marked increase of root production over the check.



Three cuttings of Tradescantia 7 weeks old showing different stages of toleration and stimulation.

No. 1 - in M/400 concentration of formaldehyde

No. 2 - in M/10,000 concentration of formaldehyde

No. 3 - in water or check.

Plate 4.



Dormant wood cuttings of *Salix fragilis* 2 weeks old in the various concentrations of formaldehyde. M/10 is on the left, and they decrease to M/200,000, with the check, on the right.



Pea seedlings in the various concentrations of formaldehyde three weeks old, showing the effects of the different concentrations on the tops and roots.

